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Gerald D. Lindsey

*Bureau of Sport Fisheries and Wildlife, Forest-Animal Unit, Olympia, Washington*

Richard M. Anthony

*Bureau of Sport Fisheries and Wildlife, Forest-Animal Unit, Olympia, Washington*

James Evans

*Bureau of Sport Fisheries and Wildlife, Forest-Animal Unit, Olympia, Washington*

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## MESTRANOL AS A REPELLENT TO PROTECT DOUGLAS-FIR SEED FROM DEER MICE

GERALD D. LINDSEY, RICHARD M. ANTHONY, and JAMES EVANS, Bureau of Sport Fisheries and Wildlife, Forest-Animal Unit, Olympia, Washington\*

ABSTRACT: Mestranol\*\* [3-Methoxy-19-nor-17 $\alpha$ -pregna-1,3,5 (10)-trien-20-yn-17-ol (C<sub>21</sub>H<sub>26</sub>O<sub>2</sub>)] was tested at 2 percent (active) as a repellent for protecting Douglas-fir (*Pseudotsuga menziesii*) seed from deer mice (*Peromyscus maniculatus*). In 5-day laboratory bioassays, deer mice consumed 61 to 66 percent fewer mestranol-treated seeds than control seeds; these results were about equal to those with a standard 0.5 percent (active) endrin seed treatment. Deer mice showed a progressive aversion to the mestranol seed treatment from 24 percent to 76 percent in 5 days. Thereafter, with minimal reinforcement, avoidance was maintained at 90 to 99 percent for 6 months. In six field trials in Washington, Oregon, and California, areas seeded with 2 percent mestranol-treated Douglas-fir seed yielded 1.6 to 5.9 times more germinants than areas seeded with control seed. In three of these areas, endrin seed treatments were included; they yielded 1.2 to 3.4 times more germinants than the mestranol treatment and 1.9 to 17.3 times more germinants than the control seed. Although the endrin treatments yielded higher numbers of germinants, the mestranol treatments in these tests generally resulted in acceptable numbers of germinants for first-year stocking. Mestranol's nontoxic, nonpersistent properties plus the aversion shown by deer mice to mestranol in our tests makes it a leading candidate as a Douglas-fir seed protectant in western United States.

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### INTRODUCTION

Since the early 1900's, seed destruction by small mammals, particularly deer mice, and to a lesser degree by birds has been one of the major limiting factors in direct seeding of conifers in the western United States (Moore, 1940; Kverno, 1964; Cone, 1967; Black, 1969; and Radwan, 1969, 1970). In the late 1940's, rodenticide baiting with 1080-treated grain alleviated rodent depredation somewhat, but it was not until the mid-1950's when an endrin treatment for conifer seed was developed that artificial seeding became commercially feasible (Spencer, 1960; Kverno, 1964; Radwan, 1969, 1970). From the late 1940's through 1972, over 1.5 million acres in Washington, Oregon, and California were seeded for conifer regeneration--almost half of the total acreage seeded in the United States (U. S. Forest Service, 1972). We estimate that 90 to 95 percent of these 1.5 million acres were seeded to Douglas-fir. Seeding in these three states peaked in 1970, when over 140,000 acres were seeded, but has since declined to less than 87,000 acres in 1972.

Some of the decline in seeding is attributable to recent constraints against using 1080 and endrin on public and private forest lands (Radwan, 1970; Evans, 1974). Although 1080 grain baits for rodent control and endrin as a conifer seed protectant are Federally registered for forest use, they are being reviewed by the Environmental Protection Agency (Ochs and Dorschner, 1974). Endrin seed treatments differing from the registered formulation (Kverno, 1964; Cone, 1967; Radwan, 1970; Derr and Mann, 1971) have caused concern. In 1973, field tests using the registered endrin formulation were started in southeastern and northwestern United States to evaluate hazards to nontarget wildlife (Evans, 1974). Regardless of the outcome of these evaluations, the use of persistent pesticides will be further restricted, and probably both 1080 and endrin will eventually be phased out of use. Unfortunately, there are no Federally registered compounds to replace them in reforestation programs.

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\*We acknowledge initial work by our coworker Larry F. Pank, who first screened mestranol at Olympia and recommended additional testing as a conifer seed treatment. We are grateful to Larry E. Johnson of the Washington Department of Natural Resources for his aid in field studies and to personnel of the U. S. Forest Service, Washington Department of Natural Resources, Simpson Timber Company of California, and Weyerhaeuser Company for assistance, materials, and study areas.

\*\*Mestranol supplied by the Syntex Corporation. Use of chemical, trade, or company names does not imply endorsement by the Federal Government.

†The registered endrin coniferous seed protectant formulation is 0.5 percent (active) endrin, 2 percent arasan, and aluminum pigment applied as a seedcoat.

In the 1960's the need for new compounds for vertebrate pest situations was recognized at the Denver Wildlife Research Center (U. S. Bureau of Sport Fisheries and Wildlife) and an extensive chemical screening program was conducted (Kverno et al., 1965). Several experimental rodenticides were selected for field testing, but for a variety of reasons, none were registered. For example, 6-aminonicotinamide proved as effective as 1080 in reducing deer mice populations (Pank and Matschke, 1972), but rapid reinvasion by deer mice indicated that frequent baiting would be necessary throughout the 3- to 6-month period that Douglas-fir seeds are exposed to rodents. Requirements for registration (Hood, 1972) and the questionable feasibility of rodenticide baiting without also treating the conifer seed (Kverno, 1964; Radwan, 1970) discouraged further investigation of this and other toxic compounds.

In 1970, we began directing most of our efforts toward searching for a nontoxic repellent to protect seed. Mestranol, although initially tested on seed in California with discouraging results (Passof's report at the Animal Damage Committee Spring Field Trip--May 17, 1968), showed good candidacy at high concentrations in our screening tests. Tests by us--and by the U. S. Forest Service (Crouch and Radwan 1971)--revealed that a 2 percent (w/w) concentration of mestranol on Douglas-fir seed equalled the endrin seed treatment in reducing seed consumption by deer mice. This report summarizes the results of additional laboratory tests and field efficacy trials.

#### CHARACTERISTICS AND USES OF MESTRANOL

Mestranol, a methylated derivative of the natural estrogen, estrone, is primarily an antifertility agent. Mixed with a progestin in pill form, it is widely used by women as an oral contraceptive. Low doses of mestranol administered by gavage, subcutaneous injection, or in food have temporarily inhibited reproduction in some species of wild vertebrates or affected fertility in their offspring (Rudel and Kind 1966; Howard and Marsh 1969; Sturtevant 1970, 1971). However, baits containing mestranol are often poorly accepted by rodents (Howard and Marsh 1969; Marsh and Howard 1969; Alsager and Yaremko 1972).

Mestranol is essentially nontoxic and nonpersistent. Carter et al., (1970) reported that continuous use (1 mg, three times daily) for 3 years produced no adverse effects in women. Research at the Denver Wildlife Research Center has indicated LD<sub>50</sub>'s exceeding 1,000 mg/kg for white rats (*Rattus* sp.), mallards (*Anas platyrhynchos*), red-winged blackbirds (*Agelaius phoeniceus*), and coturnix quail (*Coturnix coturnix*). Data from Syntex Laboratories (personal communications) show an LD<sub>50</sub> greater than 5,000 mg/kg for white mice (*Mus* sp.). Other studies have shown that once free mestranol enters a biological system--soil, microorganisms, plants, or animals--demethylation occurs quite rapidly; its half-life in these systems is less than 6 hours (Jensen et al., 1966; Sturtevant, 1970, 1971).

#### LABORATORY BIOASSAYS

##### Standard Tests

Materials and Methods: Tests were conducted with a single lot of Douglas-fir seed, untreated or treated with various combinations of the following materials: (1) crude mestranol (94 percent purity), pure mestranol (97 to 100 percent purity), and recrystallized mestranol (extracted from crude mestranol; purity unknown); (2) endrin 50-WP (50 percent wettable powder) seed protectant from Stauffer Chemical Co.; (3) Rhoplex AC-33 or AC-33X from Rohm and Haas Co., or Dow Latex 205 from Dow Chemical Co., as adhesives; and (4) mon-azral green dye (GW-749-P liquid or GT-674-D powder) from E. I. du Pont de Nemours and Co. as a coloring agent.

Four seed treatments were evaluated: untreated, control (adhesive and dye only), mestranol-treated (adhesive, dye, and mestranol), and endrin-treated (adhesive and endrin). Control and mestranol-treated seeds were first mixed with a slurry of dye and adhesive (diluted 1:10 with water), treated seeds were overcoated with mestranol while still wet, and all seeds were then scattered on plastic sheets and air-dried overnight at room temperature. Formulations contained, by weight, 0.7 percent adhesive, 0.5 percent dye, and 2.0 or 5.0 percent (active) mestranol. Endrin treatments were similarly formulated with 0.7 percent Dow Latex 205 and 0.5 percent (active) endrin.

Test animals were adult deer mice live-trapped from local Douglas-fir sites and maintained on water and laboratory chow in pens or individual cages at least 2 weeks before testing. A day or two before testing, mice were individually caged and offered 50 untreated Douglas-fir seeds. Only those that ate at least 45 seeds were used for testing. Unless otherwise stated, five individually caged new mice (not previously exposed to treated seed) were used in each bioassay. Fifty test Douglas-fir seeds were offered daily to each mouse

for 5 consecutive days; laboratory chow and water were also available in each cage. Consumption was recorded daily during the 5-day bioassay, and the mice were observed for an additional 7 days.

For germination studies, four replicate lots of 50 seeds each were placed in a seed germinator and monitored for 4 weeks.

Results: Mice ate all untreated seeds and all control seeds (formulated with combinations of adhesives and dyes); hence, reduction in consumption of treated seeds was computed from a baseline of 100 percent expected consumption. Bioassays of combinations of adhesives and dyes with pure and recrystallized mestranol on Douglas-fir seed gave similar results indicating that Rhoplex AC-33 (no longer sold because of the ban on mercury), Rhoplex AC-33X, or Dow 205 with monastral green powder or liquid did not affect the seed treatment.

Recrystallized and pure mestranol were about equal to endrin in protecting seed, and all three treatments were superior to crude mestranol (Table 1). Deer mice showed a progressive aversion to the pure and recrystallized mestranol treatments: consumption was reduced an average of 24 percent on Day 1, 64 percent on Day 2, 71 percent on Day 3, and 76 percent on Days 4 and 5. No mortalities or abnormalities were noted among mice exposed to mestranol, and they consumed normal rates of water and laboratory chow during the tests. In the three endrin tests, 40 to 80 percent of the mice died after eating treated seeds.

Table 1. Reduction in seed consumption by deer mice offered treated Douglas-fir seed. During each test, 50 treated seeds were offered daily to each of five mice for 5 days.

Treatment chemical	Percent concentration (a.i.)	Number of tests	Percent reduction in seed consumption	
			Mean	Range
Recrystallized mestranol (extracted from crude mestranol; purity unknown)	2.0	12	66	26-88
Pure mestranol (97 to 100 percent purity)	2.0	9	61	30-84
Crude mestranol (94 percent purity)	5.0	1	48	-
	2.0	2	26	13-39
Endrin	0.5	3	66	58-74

Combinations of adhesives and dye with mestranol on Douglas-fir seed and the endrin treatment did not affect germination. Germination ranged from 83 to 85 percent for untreated and control seed, from 82 to 87 percent for mestranol-treated seed, and from 81 to 86 percent for endrin-treated seed. Some germinants from untreated and mestranol-treated seed were allowed to grow until about 2 inches tall, then offered to three mice for 3 consecutive days. The mice consumed equal numbers of germinants from the two groups, suggesting little or no systemic activity of mestranol.

#### Advanced Tests

Because the 5-day bioassays showed a progressive aversion by mice to seed treated with recrystallized and pure mestranol, we conducted tests to determine if the aversive response was prolonged and how weathering affected the repellency of treated seed.

Materials and Methods: Two Douglas-fir seed treatments were formulated, as in the standard tests above: (1) with 2.0 percent recrystallize mestranol, 0.7 percent Dow Latex 205, and 0.5 percent monastral green GW-749-P dye; and (2) with 2.0 percent pure mestranol, 0.7 percent Rhoplex AC-33X, and 0.5 percent monastral green GW-749-P dye. Six 0.14-pound lots of seeds treated with Formulation 2 were spread on soil in individual 4- x 12- x 24-inch screened containers and placed outdoors for weathering in October to simulate field exposure from time of seeding to germination--normally October to about April in western Washington. Formulation 1 (unweathered) seeds were stored indoors and used as a check against the weathered seeds. (Because our supply of mestranol was limited, we could not replicate the two formulations for the weathered and unweathered treatments.) Fifteen deer mice were assigned to each formulation and were used in standard 5-day bioassays during the last week of each month, beginning in September before the Formulation 2 seeds were placed

outdoors for weathering and continuing until the seeds germinated. (Hereafter, these mice are referred to as conditioned mice.) In addition, beginning in October, the weathered seeds were tested on five new (unconditioned) mice each month as a comparison with results of standard tests.

Results: Weathered seeds began germinating in mid-March 1973, allowing only 5 months of testing; unweathered seeds were tested for 6 months. The bioassay results are given in Figure 1. Before weathering began, results of initial bioassays of both formulations showed progressive avoidance by the mice as in our standard tests--weathered group from 34 percent on Day 1 to 82 percent on Day 5; unweathered group from 36 percent on Day 1 to 78 percent on Day 5. In the October bioassays, first day avoidance by conditioned mice was high, 77 percent for weathered seed and 78 percent for unweathered seed. From Day 2 in October throughout all successive monthly bioassays, conditioned mice avoided 90 to 99 percent of both the weathered and unweathered seeds. Unconditioned mice continued to show about the same reduction in seed consumption and the same pattern of progressive aversion seen in all initial standard 5-day bioassays except for relatively poor results with seeds weathered 2 months.

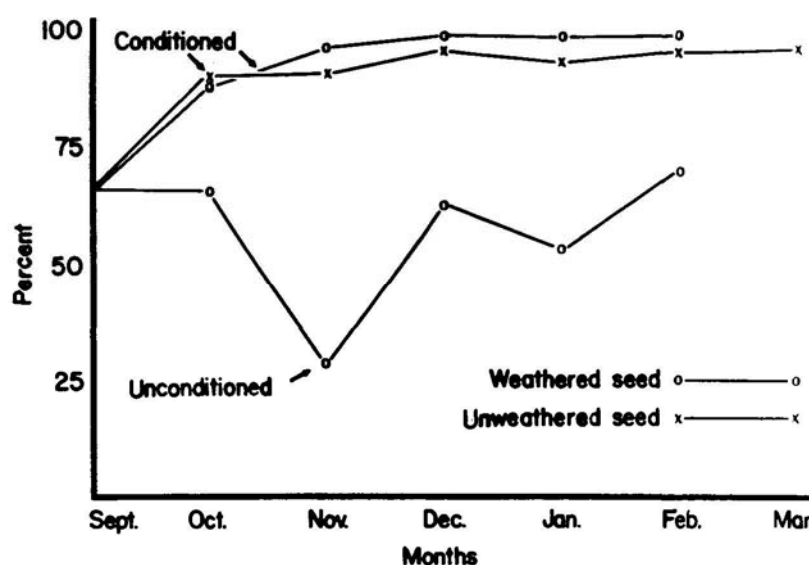


Figure 1. Results of repeated bioassays: reduction in consumption by conditioned and unconditioned deer mice of weathered and unweathered Douglas-fir seed treated with 2 percent mestranol.

These tests showed that aversion by deer mice to mestranol-treated seeds was indeed a progressive, conditioned phenomenon that could be maintained with little reinforcement. The repellency of treated seeds was not reduced by weathering for 5 months, even though the seeds were intermittently covered with snow for 18 days; 25.4 inches of precipitation fell during the period, and temperatures ranged from -3° to 64° F with 73 days of freezing temperatures. These results indicated that the 2 percent mestranol Douglas-fir seed treatment has sufficient potential for field efficacy testing.

#### FIELD TESTS

##### General Test Design

All test areas were north slopes suitable for Douglas-fir seeding. Cooperators provided seed matched for the conditions at each study site. Square 5-acre blocks were used to compare mestranol-treated seed (0.7 percent adhesive, 0.5 percent dye, and 2 percent mestranol) with control seed (adhesive and dye only) in all areas and with a standard 0.5 percent (active) endrin seed treatment (adhesive, coloring agent, and endrin 50-WP) in three

of the areas. Seed was applied at 1 pound per acre on each 5-acre block. Blocks were separated by at least 200-foot unseeded buffer zones to minimize edge-effect bias and, in some cases, to check for occurrence of natural germinants. Test-block design and area size prevented replication.

Small-mammal indices were determined before seeding and again at the beginning of germination by trapping for 2 nights with 20 Sherman live traps on a 40-foot grid in the central 1-acre quadrat in each block. Captured animals were ear tagged and/or toe clipped and released.

Germinants were counted in randomly located, circular 0.001-acre plots, 25 in the central 1-acre quadrat in each block and 25 in an adjacent unseeded area (either a separate block or a buffer zone) in each study area. All plots were examined in the spring, germinants were marked, and the plots were examined again in the summer to determine losses and count new germinants.

#### Pilot Study, 1971-72

**Study Area and Treatments:** A pilot study was conducted at Fawn Lake, Washington, at 4100-foot elevation, on a site salvage-logged following a wildfire in late 1970. Mestranol-treated seed was formulated with Dow Latex 205 adhesive, monastral green GT-674-D dye, recrystallized mestranol, and green tracerite (an inert, fluorescent tracer) for labeling mouse feces. Yellow tracerite was added to control seed. Seed was sown with a hand-operated cyclone seeder in October 1971.

**Results:** Unfortunately, faulty traps used in the pretreatment small-mammal trapping resulted in low numbers of deer mice (the only mammal captured). This problem was corrected for the spring post-treatment sampling, and again only deer mice were captured. Before treatment, 9 mice per 100 trapnights were taken on the treated block and none on the control block. Mestranol-treated seed yielded 2.2 times more germinants per acre than control seed--4,160 versus 1,880. No germinants were found in the unseeded area, and none of the feces of mice trapped in spring contained the fluorescent tracers.

#### Field Efficacy Trials, 1972-73

**Study Areas and Treatments:** Encouraged by the results of the pilot study, we then conducted extensive field evaluations on six study areas: Ah Pah Ridge and McGarvey Creek in California, Oakridge and Hebo in Oregon, and Randle and Clearwater in Washington. Elevations ranged from 500 to 4000 feet and slopes varied from less than 10 to more than 70 percent. All areas had been broadcast-burned or machine scarified with 1 year before seeding.

We compared the mestranol-treated with control seed on all areas and with endrin-treated seed on both California areas and the Clearwater area in Washington. Mestranol-treated seed was formulated with Rhoplex AC-33X adhesive, monastral green GW-749-P dye, and pure mestranol. Private and state cooperators formulated and disseminated the endrin-treated seed according to their standard procedures. All areas were sown between October 1972 and January 1973 with hand-operated cyclone seeders, except for the McGarvey Creek area and the endrin plot at Ah Pah Ridge, which were sown by helicopter.

**Results:** The relative abundance of small mammals for each study area is presented in Table 2. Deer mice were the most common small mammal captured, averaging 94 percent (100 of 106) of the pretreatment catches and 84 percent (54 of 64) of the post-treatment catches. The other small mammals caught were a few shrews (*Sorex* sp.), chipmunks (*Eutamias* sp.), and voles (*Microtus* sp.). Other seedeaters observed but not trapped were Douglas squirrels (*Tamiasciurus douglasii*), Oregon juncos (*Junco oreganus*), and varied thrushes (*Ixoreus naevius*).

The numbers of germinants per acre resulting from the various seed treatments are shown in Figure 2. No germinants were found in any of the adjacent unseeded areas. Overall, mestranol-treated seed yielded 1.6 to 5.9 times more germinants than control seed. In the three areas where the endrin treatment was included, the yield for endrin-treated seed was 1.2 to 3.4 times that of mestranol seed and 1.9 to 17.3 times that of control seed.

Table 2. Small mammal indices from live trapping results.\*

Study area	General forest type (elevation)	Small-mammal index (animals per 100 trapnights)	
		Pretreatment	Posttreatment
<u>California</u>			
Ah Pah Ridge**	Coastal (1000 ft)	12	6
McGarvey Creek**	Coastal (750 ft)	15	10
<u>Oregon</u>			
Oakridge	Cascade Mountains (2400 ft)	27	8
Hebo	Coastal (900 ft)	15	11
<u>Washington</u>			
Randle	Cascade Mountains (4000 ft)	1	6
Clearwater**	Coastal (500 ft)	10	9

\*All areas were sown with 2 percent mestranol-treated and control Douglas-fir seed.

\*\*Standard 0.5 percent endrin seed treatment also employed.

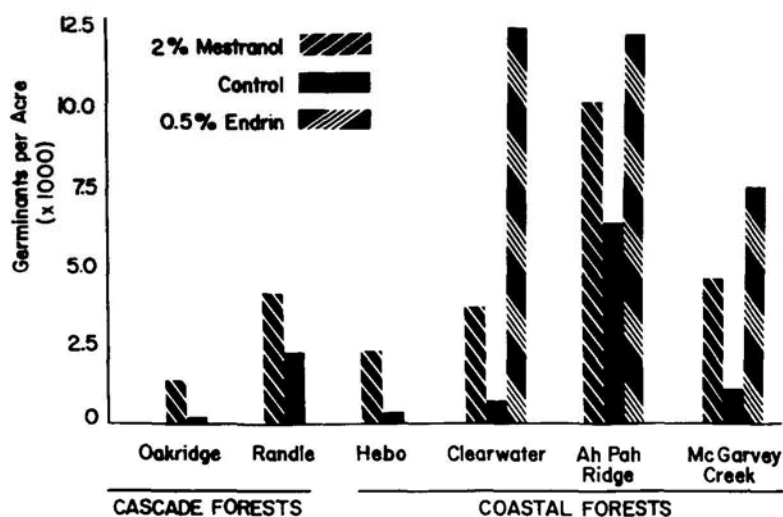


Figure 2. Numbers of germinants resulting from Douglas-fir seed treatments in six study areas. Areas were direct seeded at 1 pound per acre with mestranol-treated, control, and (in three areas) endrin-treated seed.

#### DISCUSSION AND CONCLUSIONS

We believe that our studies have demonstrated the efficacy of mestranol as a nontoxic repellent for protecting Douglas-fir seed from deer mice. Apparently, mice will eat the first few treated seeds they encounter but soon develop a conditioned aversion to mestranol that can be maintained for a prolonged period by occasional reinforcement or exposure. This same pattern of conditioned bait aversion has occurred--and has been considered a disadvantage--in studies of mestranol as a reproductive inhibitor. Bait formulations of 0.005 to 0.05 percent mestranol have inhibited reproduction in several species of rodents but have also produced aversion (Howard and Marsh, 1969; Marsh and Howard, 1969). We had to use the much higher concentration of 2 percent to achieve the aversive effect because deer mice hull Douglas-fir seeds, eating the female gametophyte and discarding the seedcoat, which carries most of any topically applied chemical.

In laboratory studies, crude mestranol was rather ineffective, but pure and recrystallized mestranol was about as effective a seed protectant as endrin, did not interfere with germination, and withstood weathering quite well. In field trials the endrin treatments yielded more germinants than the mestranol treatments. Nevertheless, mestranol-treated seed consistently yielded more germinants than control seed and gave levels of germinant production generally considered acceptable in seeding operations, except at Oakridge, Oregon, where direct seeding has seldom been successful regardless of seed treatment.

In situations where Douglas-fir or other seed are exposed to depredation for long periods, we believe that seed treatments are essential. An effective seed treatment should obviate the need for rodenticide baiting, which rarely gives more than short-term protection. Because mestranol is nontoxic and non-persistent, and the 2 percent treatment appears to adequately protect Douglas-fir seed from deer mice, we are proceeding with the compound's development and have begun studies of mestranol residues in soil, water, and vegetation to support a petition for Federal registration.

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